

DTIC FILE COPY

②

AD-A230 232

Best Available Copy

NICOTINIC CHOLINERGIC RECEPTORS IN RAT BRAIN

Annual Report Number 2

Kenneth J. Kellar

May 13, 1985

DTIC  
ELECTE  
DEC 26 1980  
S B D  
Co

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701-5012

Contract Number: DAMD17-83-C-3113

Georgetown University Medical Center

Washington, DC 20007

Approved for Public Release; Distribution Unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

20030213000

20 10 21 027

## REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION Georgetown University School of Medicine	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS (City, State, and ZIP Code) Washington, DC 20007-2195		7b. ADDRESS (City, State, and ZIP Code)	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-83-C-3113	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 61102A	PROJECT NO. 3M1-61102BS11
		TASK NO. EE	WORK UNIT ACCESSION NO. 053
11. TITLE (Include Security Classification) (U) Nicotinic Cholinergic Receptors in Rat Brain			
12. PERSONAL AUTHOR(S) Kenneth J. Kellar, Ph.D.			
13a. TYPE OF REPORT Annual Report	13b. TIME COVERED FROM 84/5/1 TO 85/4/30	14. DATE OF REPORT (Year, Month, Day) 5/13/85	15. PAGE COUNT 9
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
06	01		
06	15		
		Brain, Nicotinic receptors, Acetylcholine, Nicotine binding, Behavior, <i>etc.</i>	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) We have conducted experiments to determine if [ <sup>3</sup> H]acetylcholine ([ <sup>3</sup> H]ACh) nicotinic recognition sites are located presynaptically on catecholamine and/or serotonin axons. Lesions of these axons by intraventricular injections of neurotoxins resulted in marked decreases in [ <sup>3</sup> H]ACh binding sites in the striatum and hypothalamus, but not in the cortex or thalamus. These results indicate that [ <sup>3</sup> H]ACh nicotinic binding sites are located on catecholamine and serotonin axons in specific areas of the brain. In other experiments, we determined that repeated administration of nicotine results in enhanced behavioral responses to a subsequent injection of nicotine, and that there appears to be a correlation between the enhanced response to nicotine and increased [ <sup>3</sup> H]ACh binding sites in cerebral cortex.			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian		22b. TELEPHONE (Include Area Code) 301/663-7325	22c. OFFICE SYMBOL SGRD-RMI-S

## FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (DHHS, PHS, NIH Publication No. 86-23, Revised 1985).

Accession For	
NPIS GRA&I	<input checked="checked" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Avail and/or	
Dist	Special
A-1	

## TABLE OF CONTENTS

	Page
Foreword	1
Presynaptic location of [ $^3$ H]ACh recognition sites in rat brain	3
Behavioral effects of repeated administration of nicotine	6
Correlation between behavioral effects and [ $^3$ H]ACh binding sites after repeated administration of nicotine	6
Tables and Figures	
Table 1. [ $^3$ H]ACh binding in brain regions from control rats and from rats lesioned with 6-OHDA or 5,7-DHT injected intraventricularly.	4
Table 2. Binding constants of [ $^3$ H]ACh in striatum from control, 6-OHDA, and 5,7-DHT lesioned rats.	5
Figure 1. Locomotor activity in the first 10 min after injection of 0.2 mg/kg of nicotine on each successive day.	7
Figure 2. Comparison of the behavioral effects of nicotine and nicotinic receptor binding under various conditions.	8
Distribution List	9

During the past year we have conducted studies to:

1. Determine if the nicotinic cholinergic agonist binding site is located presynaptically on catecholamine axons where it might be involved in the regulation of release of catecholamine neurotransmitters.
2. Determine if the nicotine-induced increase in nicotinic receptors measured by binding studies results in increased behavioral responses to nicotine.

This report describes the methods that we used and the results of those studies.

1. *Presynaptic location of [<sup>3</sup>H]acetylcholine recognition sites in rat brain.* To determine if [<sup>3</sup>H]acetylcholine ([<sup>3</sup>H]ACh) recognition sites are located on catecholamine or serotonin axons in rat brain, male Sprague-Dawley rats (250-300 g) were anesthetized with equithesin and placed in a stereotaxic instrument. Either 6-hydroxydopamine (6-OHDA; 250 µg) or 5,7-dihydroxytryptamine (5,7-DHT; 250 µg) dissolved in 10 µl saline containing 0.1% ascorbic acid was then infused intraventricularly over a 5 min period. These neurotoxins selectively lesion catecholamine and serotonin axons, respectively. Control rats were infused similarly with vehicle. The rats infused with 5,7-DHT were pretreated with desipramine (25 mg/kg) 30 min before intraventricular infusions to ensure selective lesions of serotonin neurons. All rats were sacrificed by decapitation 7-10 days after surgery and the brains were dissected, frozen on dry ice, and stored at -80°C until assayed for nicotinic cholinergic receptors using the [<sup>3</sup>H]ACh binding assay (Schwartz et al., 1982).<sup>1</sup> The extent and selectivity of the lesions were assessed by measuring the content of dopamine and serotonin in the striatum by high performance liquid

---

<sup>1</sup>Schwartz, R.D., R.M. McGee, Jr. and K.J. Kellar: Nicotinic cholinergic receptors labeled by [<sup>3</sup>H]acetylcholine in rat brain. *Mol. Pharmacol.* 22: 56-62 (1982).

chromatography (HPLC) with electrochemical detection and by measuring the uptake of [<sup>3</sup>H]norepinephrine and [<sup>3</sup>H]serotonin in synaptosomes prepared from fresh hypothalamus. These measurements indicated that 6-OH<sup>+</sup> produced a selective lesion of catecholamine axons and that 5,7-DHT produced a selective lesion of serotonin axons. Statistical analyses of data were carried out using Duncan's new multiple range test.

Following the 6-OHDA lesions of catecholamine axons, [<sup>3</sup>H]ACh binding in the striatum and hypothalamus was decreased by 30 percent and 60 percent, respectively (Table 1). Similarly, following 5,7-DHT lesions of serotonin axons, [<sup>3</sup>H]ACh binding in the striatum and hypothalamus was decreased by 30 percent and 43 percent, respectively (Table 1). In contrast to the striatum and hypothalamus, binding in the cortex and thalamus was not significantly affected by either of these lesions (Table 1).

Table 1. [<sup>3</sup>H]ACh binding in brain regions from control rats and from rats lesioned with 6-OHDA or 5,7-DHT injected intraventricularly. Assays were conducted using 10 nM [<sup>3</sup>H]ACh. Values are means  $\pm$  SEM from the number of animals indicated in parentheses<sup>2</sup>.

Brain area	Control	Specific binding (fmol/mg protein)	
		6-OHDA	5,7-DHT
Cortex	38.4 $\pm$ 2.6 (4)	36.2 $\pm$ 1.9 (4)	36.0 $\pm$ 2.1 (4)
Thalamus	53.7 $\pm$ 1.2 (4)	56.6 $\pm$ 2.1 (4)	54.0 $\pm$ 2.1 (4)
Striatum	47.3 $\pm$ 2.2 (8)	33.0 $\pm$ 3.2 (6)	33.2 $\pm$ 1.7 <sup>a</sup> (7)
Hypothalamus	41.5 $\pm$ 3.5 (5)	16.9 $\pm$ 1.1 <sup>b</sup> (6)	23.6 $\pm$ 3.6 <sup>b</sup> (6)

<sup>a</sup>p < 0.01; <sup>b</sup>p < 0.001.

<sup>2</sup>Please note that [<sup>3</sup>H]ACh binding values are expressed on a per mg protein basis rather than the per mg wet weight basis used in Annual Report No. 1.

To determine whether there was a loss of binding sites or a change in affinity of the sites for [ $^3\text{H}$ ]ACh, saturation binding of [ $^3\text{H}$ ]ACh was measured in the striatum. Table 2 shows that following both lesions, the decreased binding was due to a loss of binding sites (decreased  $B_{\text{max}}$ ) with no significant change in affinity ( $K_d$ ).

Table 2. Binding constants of [ $^3\text{H}$ ]ACh in striatum from control, 6-OHDA, and 5,7-DHT lesioned rats. Striatal homogenates were incubated with [ $^3\text{H}$ ]ACh (1-30 nM) for 40 min at 0°C and then filtered through Whatman GF/C filters. Values are the mean  $\pm$  SEM from 3 experiments.

	<u>Control</u>	<u>6-OHDA</u>	<u>5,7-DHT</u>
$K_d$ (nM)	13.0 $\pm$ 1.4	11.8 $\pm$ 1.3	10.3 $\pm$ 0.3
$B_{\text{max}}$ (fmol/mg protein)	108.4 $\pm$ 7	80.9 $\pm$ 1.8*	84.5 $\pm$ 5.5*

\*p < 0.05.

The results of these studies indicate that nicotinic cholinergic recognition sites are located on catecholamine and serotonin axons in the striatum and in the hypothalamus but not in the cortex or thalamus. These receptors may be involved in the modulation of dopamine, norepinephrine, or serotonin release. Although nicotine has been reported to stimulate catecholamine and serotonin release from brain slices, in most of the reported studies, very high concentrations of nicotine were utilized and the pharmacology of the effects was not investigated. Nevertheless, the presence of nicotinic cholinergic recognition sites on catecholamine and serotonin axons in the striatum and hypothalamus suggest that regulation of release of neurotransmitters may be one function of these sites. In addition, the presence of these

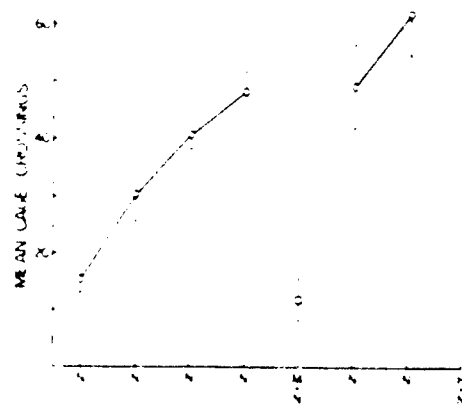
recognition sites on axons in the hypothalamus suggests the possibility that they may also be involved in the release of hypothalamic hormones that regulate pituitary function.

2. *Behavioral effects of repeated administration of nicotine.* To determine whether there was a correlation between the nicotine-induced increase in [ $^3\text{H}$ ]ACh binding sites and behavioral effects of nicotine, the effects of repeated administration of nicotine on nicotine-induced locomotor activity were measured and compared to changes in [ $^3\text{H}$ ]ACh binding sites in brain.

Male Sprague-Dawley rats (250-300 g; approximately 3 months of age) were injected subcutaneously with nicotine once daily for up to 8 days. Following each injection, the rats were tested in identical cages equipped with two infrared photocell beams which were monitored by a microcomputer. Alternately breaking the two beams was counted as a cage-crossing. The rats were allowed to habituate to the photocell cages for 1 hour before each injection.

In the first experiment, the rats were injected and tested each day with 0.2 mg/kg nicotine. On two days, either mecamylamine (1 mg/kg), a nicotinic antagonist that crosses the blood-brain barrier, or hexamethonium (2 mg/kg), a nicotinic antagonist that does not readily enter the brain, was injected 20 min before the nicotine injection. The results of these behavioral studies are shown in Figure 1. Repeated treatment with nicotine induced progressively increased locomotor responses. These responses to nicotine were completely blocked by mecamylamine, but were unaffected by hexamethonium.

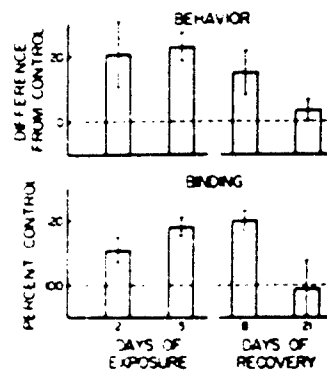
Figure 1



Locomotor activity in the first 10 min after injection of 2 mg/kg of nicotine on each successive day. On the fifth day the effect of nicotine was antagonized by an injection of 2 mg/kg of mecamylamine (M = N). Hexamethonium (2 mg/kg) was administered prior to nicotine on the eighth day (H = N) (n = 7).

In the second experiment, nicotine (0.2 mg/kg) or saline was administered for 2 days or 5 days before measuring nicotine-induced locomotor activity. Other rats were treated with nicotine or saline for 5 days and the behavioral measurements were made either 8 days or 21 days after the last injection to test for recovery. Following the behavioral tests, the rats were sacrificed and the brains were dissected and frozen until assayed for nicotinic cholinergic receptor binding sites. The results of these experiments are shown in Figure 2.

Figure 2



Comparison of the behavioral effects of nicotine and nicotinic receptor binding under various conditions: after 2 or 5 days of exposure to 0.2 mg/kg of nicotine or, after 5 days of exposure to 0.2 mg/kg of nicotine and then either 8 or 21 days without nicotine ( $n = 5$  for 2 and 5 day exposure groups;  $n = 6$  for 8 and 21 recovery groups).

Both 2 days and 5 days of nicotine treatment resulted in an increase in nicotine-induced locomotor behavior. This increased response to nicotine persisted for at least 8 days after the last of 5 daily nicotine injections, but the response returned to control levels 21 days after the last nicotine injection. [ $^3$ H]ACh binding sites in the cerebral cortex were increased after both 2 days and 5 days of nicotine treatment. This increase in binding was still seen 8 days after the last of 5 daily nicotine injections, but like the behavioral response, the binding returned to control values 21 days after the last injection. Thus, there appears to be at least some correspondence between nicotine-induced increases in a behavioral response and increases in nicotinic cholinergic recognition sites labeled by [ $^3$ H]ACh.

## DISTRIBUTION LIST

1 copy	Commander U.S. Army Medical Research and Development Command ATTN: SGRD-RMI-S Fort Detrick, Frederick, Maryland 21702-5012
5 copies	Commander U.S. Army Medical Research and Development Command ATTN: SGRD-PLF Fort Detrick, Frederick, Maryland 21702-5012
2 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-FDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of the Health Sciences, U.S. Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100